OCCURRENCE OF ACRYLAMIDE IN PORTUGUESE BREAD

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Abstract

Acrylamide is known for its potential health hazards. The aim of this study was to analyze the acrylamide content of confectioned bread in fifty four different pastries.

Acrylamide levels obtained were high, ranging between 59 and 1273 µg/kg. Only two bakeries had lower quantities of acrylamide than the indicative value of EFSA, 150 µg/kg. These differences can be associated with the products confection process, but also with the use of different recipes bread.

Keywords: Acrylamide, Occurrence, UPLC-MS, EFSA

1. INTRODUCTION

Acrylamide is a colorless, non-volatile crystalline solid. This vinyl compound dissolves in water, alcohol, and other polar solvents.

Acrylamide is mainly used as an intermediate in the reaction of polyacrylamide formation [1]. It is presented in cooked foods and cigarette smoke, however, the occurrence of acrylamide in foods was only firstly reported in 2002 [1].

In 1994, the International Agency for Research on Cancer (IARC) classified acrylamide as a carcinogen in animals and probably to humans (Group 2A) [2].

Later, acrylamide was considered a neurotoxic substance. This substance causes injuries to the peripheral nervous system and affects the central nervous system in cases of overexposure [1] [3].

There are three pathways for the formation of acrylamide in processed foods [4][5]. The route of lipids, secondary route, is the thermal decomposition of lipids which results in large amount of acrolein. Acrolein´s oxidation generates acrylic acid which, in the presence of ammonia, originates acrylamide. The other secondary pathway, the carnosine, is the reaction of pyrolysis of carnosine forming acrylic acid. As in the case of lipid pathway, acrylic acid in the presence of ammonia originates acrylamide. Finally, the main route for acrylamide formation in foods is a part of the Maillard reaction with free amino acid (asparagine) and reducing sugars. The reaction of these precursors results in a Schiff base which undergo decarboxylation gives the 5-1-oxazolidine. Subsequently, the decarboxylated product could decompose either to acrylamide or 3-aminopropionamide (3-APA). The excess sugar could also enhance the conversion from 3-APA to acrylamide [6][7].

People are exposed to different amounts of acrylamide mainly through the diet, since this contaminant is formed mostly in potato, cereal and coffee. So, it became important to assess the occurring levels in various processed foods. However, until this date EFSA did not set maximum limits, but stated indicative values for various food groups such as cereals (Table 1) [8].

Data occurrence on cereals is published. M. Cengiz, C. Gündüz reported acrylamide values in bread 225 µg/kg and 495 µg/kg in cookies [9]. In Finland, S. Eerda, K. Hollebekkers, Hallikainen A. and K. Peltonen, obtained acrylamide levels in bread and sweet biscuits of 645 µg/kg and 310 µg/kg [10], respectively, but a study in Belgium showed lower results in bread with 34 µg/kg and biscuits with 154 µg/kg [11].

To this date, in Portugal, occurrence studies in cereal group weren´t found. Therefore, the aim of this study is to assess the levels of acrylamide in bread confectioned in several pastries.

Table 1. Indicative values set by the European Commission for acrylamide content in cereal-based foods [8]

Foodstuf	Indicative Values (µg/kg)
Soft bread	
a) Wheat based bread	80
b) Soft bread other than wheat based bread	150
Breakfast cereals	
- Bran products and whole grain cereals	400
- Wheat and rye based products	300
- Maize, oat spelt, barley and rice based products	200
- Biscuits and wafers	500
- Crackers	500
- Crispbread	450
- Gingerbread	1000
- Products similar	500
Biscuits and rusks for infants and young children	200
Processed cereal based foods for infants and young children	50

2. MATERIALS AND METHODS

2.1 Sampling and sample preparation

The samples analyzed included several bread varieties, which were collected randomly in 54 pastries. 2 g of homogenized sample were weighed into a centrifuge tube. It was added 20 ml water/ formic acid (0.1%), stirred in a vortex for 2 minutes and then in an oscillating shaker for 30 min at 70 oscillations per minute. Then, it was centrifuged at 10,000 rpm for 15 minutes.

Oasis HLB SPE cartridges were conditioned with 3.5 mL of methanol and equilibrated with 3.5 mL of acidified water. 1.5 ml of the sample was loaded and eluted with 3 mL of acidified water to a flask of 10 mL and the volume was completed with acidified water.

2.2 Chemicals and reagentes

For this study, the following reagents were used: Acetonitrile (Merck grandient grid is liquid chromatography), formic acid (Group Carlo Erba Reagents, 99% for analysis) Methanol (Merck, hypergrade for LC-MS) and ultrapure type 1 water (captured from a Milli-Q water purification system.

The standard used was the standard of acrylamide (99%) Dr. Ehrenstorfer GmbH).

2.3 Chemical Standards

The pattern of acrylamide was dissolved in water/formic acid (0.1%) at a concentration of 90000 µg/L. dilutions were performed to give six concentrations, 0, 1, 10, 50, 150 and 250 µg/L.

2.4 LC-MS analysis

Sample analysis was conducted with UPLC coupled to an MS with electrospray ionization source (ESI). Samples were separated by UPLC BEH C18 column (2.1 × 50 mm).

The mobile phase was 90% water and 10% acetonitrile with a flow rate of 0.2 ml/min for 3 minutes. The sample injection volume was 10 µL. The positive electrospray ionization source had the following conditions: capillary voltage of 3 kV, the cone voltage 29 V, source temperature 120 ° C, desolvation gas temperature 350 ° C, desolvation gas flow of 5 L/hr cone gas flow at 30 L/hr and the pressure of the collision gas was 3×10^{-3} mbar.

2.5 Traceability

Equipment used during experiments was calibrated according to approved calibration procedures and external standards traceable to national measurements standard, when available.

All volumetric glassware belongs to Class A. Certified reference material ERM –BD273 (toasted bread powder), was purchase from IRMM.

1.2 Quality Assurance

Laboratory has participated in several PT schemes launched by accredited provider FAPAS to guarantee the laboratory performance.

All the assays were carried out under quality assurance procedures framed by ISO/IEC 17025.

1.3 Statistical analysis

Statistical analysis of the data was performed in STATISTICA using ANOVA in order to test the difference in acrylamide levels.

2. RESULTS AND DISCUSSION

In order to evaluate the performance of laboratory to carry out analytical method, a proficiency test by Food Analysis Performance Assessment Scheme (FAPAS), was made, obtaining a z-score of 0.6.

The results of the samples are shown in Table 2. The bakeries 31 (1258.99 µg/kg), 5 (1235.81 µg/kg), 26 (1208.28 µg/kg) and 14 (1182.42 µg/kg) have the highest acrylamide content in bread. These values are higher than those found in Czech bread, 16 µg/kg [12].

Comparing this study to the acrylamide levels found in bread by M. Cengiz, C. Gündüz, 225 µg/kg [9], it is verified that, in Portugal, there are bakeries with lower values, such as 16, 53 and 50. However these bakeries have higher concentrations when compared to a study conducted in Germany, in which a value of 34 µg/kg for bread was found [11].

In a study of Finnish food, results in a range between 68-1480 µg/kg for bread were obtained [10]. The acrylamide values obtained in this study are in the same range, with the exception of bakery 16 which is below the minimum value obtained by Finland.

Comparing the results with an earlier study [13], 625 µg/kg, it appears that the majority of bread produced in under studied have higher amounts of acrylamide than in the previous study. This demonstrates that no measures for reducing acrylamide in bread have been applied yet.

The results obtained in this study are higher than the recommended value of EFSA for fresh bread, 150 µg/kg, with the exception of bakery 16 and 53 which are below [8].

The differences between bakeries may be related to the processing mode used for the confection of the product under study [14].

3. CONCLUSIONS

Bread is one of the products that most contribute to the exposure of the population to acrylamide.

Table 2. Acrylamide levels in bread collected in several bakeries

Place	Acrylamide (µg/kg)	Place	Acrylamide (µg/kg)
1	328.78 ± 3.15	28	1168.95 ± 69.48
2	786.86 ± 2.24	29	843.37 ± 10.25
3	247.50 ± 18.03	30	723.55 ± 9.26
4	1159.67 ± 5.39	31	1258.99 ± 11.67
5	1235.81 ± 52.29	32	749.57 ± 47.1
6	741.17 ± 35.26	33	325.96 ± 6.22
7	1149.82 ± 3.31	34	209.53 ± 14.18
8	536.08 ± 44.48	35	751.27 ± 35.37
9	638.34 ± 23.53	36	168.31 ± 12.92
10	505.98 ± 2.29	37	319.38 ± 10.15
11	712.97 ± 65.94	38	368.45 ± 9.21
12	645.94 ± 25.72	39	205.45 ± 18.37
13	269.40 ± 5.22	40	252.83 ± 13.58
14	1182.42 ± 14.05	41	278.30 ± 20.29
15	834.49 ± 18.48	42	210.71 ± 13.17
16	59.94 ± 4.02	43	216.23 ± 17.24
17	807.88 ± 2.07	44	161.98 ± 6.79
18	715.67 ± 2.31	45	252.96 ± 21.30
19	344.61 ± 12.43	46	204.84 ± 16.54
20	937.31 ± 15.12	47	201.46 ± 13.13
21	1105.38 ± 23.56	48	160.37 ± 12.70
22	546.18 ± 2.99	49	176.86 ± 16.56
23	579.97 ± 7.60	50	159.71 ± 5.80
24	1273.09 ± 28.91	51	387.83 ± 26.87
25	783.38 ± 17.29	52	195.42 ± 4.76
26	1208.28 ± 48.19	53	119.7 ± 4.14
27	851.10 ± 5.91	54	749.57 ± 47.10

Thus, this study was determined acrylamide content in confectioned bread in several bakeries located throughout the country.

The amounts of acrylamide found in selected breads are higher than the data reported by EFSA, 36-49 µg/kg.

It was also observed that only bread produced by two bakeries have amounts of acrylamide below indicative value of EFSA 150 µg/kg.

In conclusion, we observed significant differences between the samples of bread from various bakeries. These differences may be associated with different confection methods such as fermentation time and temperature. Also, the use of additives can interfere in the formation of this contaminant in the basic products.

It is essential to apply reduction methods of acrylamide in baked goods, due to given the values obtained in this study being above the indicative value of EFSA.

4. ACKNOWLEDGEMENTS

The authors appreciate the financial support of Project ELEMENTARIA – 2013DAN850. Susana Jesus acknowledges cooperation agreement between FCT-UNL and INSA under the frame of student exchange.

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